Remarks

With this amendment, claims 22-31, 33-44, 46-57, 59, 60, and 69-80 are pending. Claims 32, 45, 58, and 61-68 have been canceled. Claims 22, 35, and 48 have been amended. Claims 69-80 are newly added.

Support for the amendments to claims 22, 35, and 48 can be found in the specification at p. 16, lines 5-7, and p. 18, lines 7-9. Further support for the amendments to claims 22, 35, and 48 can be found in currently canceled claims 32, 45, and 58, respectively, and in the specification at p. 16, Table 1. Support for newly added claims 69, 73, and 77 can be found in the specification at p. 16, Table 1. Support for newly added claims 70, 74, and 78 can be found in the specification at p. 16, Table 1. Support for newly added claims 71, 75, and 79 can be found in the specification at p. 17, Table 3. Support for newly added claims 72, 76, and 80 can be found in the specification at p. 19, Table 6.

Applicants reserve the right to submit canceled claims in a continuation application.

I. 35 U.S.C. 112, Second Paragraph

Claims 61-68 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite of failing to particularly point out and distinctly claim the subject matter which applicants regard as the invention.

The Office rejected claims 61-68 for reciting the term "mobilized," asserted by the Office to be vague and indefinite. Without conceding to the merits of the Office's rejection, applicants have canceled claims 61-68 with this amendment, thereby rendering this rejection moot.

II. 35 U.S.C. 102(e) Rejection

A. Blumberg et al. (U.S. Patent No. 5,763,215 and EP 191,827)

Reconsideration is requested of the rejection of claims 61-63 and 66-67 under 35 U.S.C. 102(e) as being unpatentable over Blumberg et al. (U.S. Patent No. 5,763,215) and Blumberg et al. (EP 191,827).

Without conceding to the merits of the Office's rejection, applicants have canceled claims 61-63 and 66-67 with this amendment. Accordingly, this rejection is rendered moot.

B. Pedersen et al. U.S. Patent No. (5,783,413)

Reconsideration is requested of the rejection of claims 61-64 and 66-67 under 35 U.S.C. 102(e) as being unpatentable over Pedersen et al. (U.S. Patent No. 5,783,413).

Without conceding to the merits of the Office's rejection, applicants have canceled claims 61-64 and 66-67 with this amendment. Accordingly, this rejection is rendered moot.

II. 35 U.S.C. 103(a) Rejection

Reconsideration is requested of the rejection of claims 22-68 under 35 U.S.C. 103(a) as being unpatentable over Blumberg et al. (U.S. Patent No. 5,763,215) and Pedersen et al. (U.S. Patent No. 5,783,413) in view of Prescott et al. (1975) and Prescott et al. (1966).

As amended, claim 22 is directed to a method of removing an alanyl residue from the N-terminal region of a polypeptide. The method comprises expressing a polypeptide having an alanyl residue in the N-terminal region, forming a solution containing the expressed polypeptide and contacting the solution with

immobilized *Aeromonas proteolytica* aminopeptidase to cleave the alanyl residue from at least 48% of the polypeptide molecules in the solution.

As amended, claim 35 is directed to a method of removing an N-terminal alanyl residue from a polypeptide. The method comprises expressing a polypeptide having an N-terminal alanyl residue, forming a solution containing the expressed polypeptide and contacting the solution with immobilized *Aeromonas proteolytica* aminopeptidase to cleave the N-terminal alanyl residue from at least 48% of the polypeptide molecules in the solution.

As amended, claim 48 is directed to a method of removing an N-terminal alanyl residue from a recombinantly expressed polypeptide having an N-terminal alanyl residue. The method comprises forming a solution containing the expressed polypeptide and contacting the solution with immobilized *Aeromonas proteolytica* aminopeptidase to cleave the N-terminal alanyl residue from at least 48% of the polypeptide molecules in the solution.

Blumberg et al. disclose what they generally characterize as a method of removing N-terminal amino residues from eucaryotic polypeptide analogs. The focus of the disclosure is upon the removal of N-terminal methionine residues. For use in this method, they suggest using any of a number of alternative aminopeptidase enzymes. Blumberg et al. passingly note that the aminopeptidase can be, *inter alia*, bound to a solid support,[1] although none of the examples implement such a method. In a

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[1] Blumberg et al., U.S. Patent No. 5,763,215 at column 4, lines 42-44.

preferred embodiment of the invention, the aminopeptidase used is *Aeromonas* aminopeptidase.[2]

In Example X, Blumberg et al. incubated a dissolved mixture of *Aeromonas* aminopeptidase and Cu_2-Zn_2 Human Superoxide Dismutase which has an N-terminal alanine, achieving a removal of only 5.2% of the N-terminal alanine, characterizing it as a **non-removal**.[3]

Blumberg et al. also incubated a dissolved mixture of *Aeromonas* aminopeptidase with Met-hGH and Met-Leu-hGH in Examples I and VI, respectively, disclosing the results in Tables I and V, respectively. The removal of alanine in each example is disclosed as being negligible[4]. Moreover, Blumberg et al. state that polyacrylamide gel electrophoresis of the resulting hGH product reveals no detectable degradation of the hGH beyond the removal of the Met and Met-Leu residues.[5]

Blumberg et al. also propose that an aminopeptidase may remove an alanine from the N-terminal region of the alanine form of bGH.[6] Significantly, however, Blumberg et al. did not state that the alanine ***would*** be removed; they merely suggested that it

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[2] Blumberg et al., U.S. Patent No. 5,763,215 at column 2, line 66 through column 3, line 3, emphasis added.

[3] Blumberg et al., U.S. Patent No. 5,763,215 at column 16, lines 5-50.

[4] *See*, Blumberg et al., U.S. Patent No. 5,763,215 at column 9, Table I footnotes, and at column 14, Table V footnotes.

[5] Blumberg et al., U.S. Patent No. 5,763,215 at column 9, lines 13-15 and column 14, lines 42-43.

[6]Blumberg et al., U.S. Patent No. 5,763,215 at column 5, lines 43-57, emphasis added.

may also be removed. Likewise, Blumberg et al. failed to disclose which of the number of aminopeptidases they propose ***may possibly*** remove this alanine.

Accordingly, Blumberg et al. do not disclose the removal of an N-terminal or N-terminal region alanyl residue from at least 48% of the polypeptide molecules contacted by the aminopeptidase and immobilization of the aminopeptidase as required by the claims.

Pedersen et al. disclose the use of aminopeptidases to remove N-terminal residues or combinations of residues. Pedersen et al. passingly note that *Aeromonas* aminopeptidase, among other aminopeptidases, could be used to achieve good results. They further disclose that *Aeromonas* aminopeptidase can be used to remove a single amino acid from the N-terminus of a protein or polypeptide, citing EP 191 827 (Blumberg et al.) and EP 489 711, asserting that under the appropriate conditions "amino acid removal will commence and continue unless or until (1) the amino group of the N-terminal is blocked, (2) the site of removal is on the N-terminal side of proline, or (3) the N-terminal amino acid is glutamic or aspartic acid."[7] Such disclosures notwithstanding, the only references within Pedersen et al. to the removal of an N-terminal alanyl residue are Examples 8 and 9. However, neither Example 8 nor Example 9 disclose the use of *Aeromonas* aminopeptidase.

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[7] Pedersen et al, U.S. Patent No. 5,783,413 at column 3, lines 8-12.

As discussed in Amendment C,[8] applicants assert that the use of the phrase " . . .(Biotinylated-AAP . . .). . ." in Example 9 is a typographical error, demonstrated by the use of the phrase "Biotin-APP" twice within the same example and the disclosure within the Methods and Materials section[9] of the preparation of ***each and every enzyme used in the Examples***. Noticeably missing from that description is a description of the preparation of AAP - ***a description that one would expect to find if indeed AAP were used in Example 9***.

While the Office asserts in the present Office action that "AAP is so well known and its method of preparation is so well known that it was not necessary to provide a method of making" AAP,[10] such a conclusion does not naturally extend from the disclosure contained in Pedersen et al. Specifically, the preparation of DAP I and GCT as outlined in the Methods and Materials section of Pedersen et al. are disclosed therein to be based upon methods taught in references published in 1966 and 1965, respectively - almost 30 years prior to the priority date of Pedersen et al. and within about one year of the publication of the earliest Prescott et al. reference cited against applicants.[11] It would seem therefore, that DAP I and GCT, as

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[8] Amendment C, dated April 7, 2003, at p. 11, line 3, through p. 12, line 5.

[9] Pedersen et al. U.S. Patent No. 5,783,413 at column 6, lines 15-50.

[10] *See*, present Office action dated June 17, 2003, at p. 6, lines 2-3.

[11] The Office cited Prescott et al., *Archives of Biochemistry and Biophysics*,117:328-336, published in 1966, in the present Office action.

well as the methods of producing each, are also well known, thereby, according to the Office's asserted theory, obviating any need for Pedersen et al. to provide a method of preparation for each. However, the methods of preparing both are nevertheless specifically outlined in the Methods and Materials section. Accordingly, it is more likely that the method of preparing AAP was not disclosed in Pedersen et al. because AAP was not used in any of the examples in Pedersen et al, including Example 9. These points taken together, one would be left to conclude that the parenthetical "Biotinylated-**AA**P" in Example 9 is a typographical error and that Biotin-APP, rather than Biotin-AAP, was the aminopeptidase used in Example 9. Accordingly, there is no disclosure within Example 9, nor anywhere else in Pedersen et al., of the use of *Aeromonas* aminopeptidase to remove an N-terminal or N-terminal region alanyl residue from at least 48% of the polypeptide molecules contacted by the aminopeptidase and immobilization of the aminopeptidase as required by the claims.

Prescott et al. (1975) disclose that the "catalytic activity of *Aeromonas* aminopeptidase toward the amides of all the common amino acids has been determined,"[12] citing to Wagner et al, *J. Biol. Chem.*, 247:1208 (1971)[13], and disclosing in Table II the data obtained by Wagner et al.[14] Most notably, footnote b of

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[12] Prescott et al. (1975), p. 537-538.

[13] This reference was cited by the Office in the present Office action, dated June 17, 2003, with respect to the 35 U.S.C. 103(a) rejection of now canceled claims 61-63 and 66-67.

[14] The data obtained by Wagner et al. is disclosed in Wagner et al. in Table I, p. 1209. The same data is disclosed in Prescott et al. in Table II, p. 539. Wagner et al. disclose the influence exerted by the NH_2-terminal and penultimate residues of

Table II discloses that "amino acid amides that the enzyme [*Aeromonas* aminopeptidase] failed to hydrolyze when tested at 20 mM concentration were alaninamide . . ."[15] Additionally, Prescott et al. (1975) disclose that less specificity is evident and generally high hydrolytic rates are observed with enzyme-to-substrate ratios of 1:2500, having proved effective for most of the oligopeptides tested.[16] This data is contained in Table III.[17] Although several of the oligopeptides tested contained alanine residues, none of the alanine residues were removed by the aminopeptidase. Accordingly, Prescott et al. (1975) do not disclose the removal of an N-terminal or N-terminal region alanyl residue from at least 48% of the polypeptide molecules contacted by the aminopeptidase and immobilization of the aminopeptidase as required by the claims.

Prescott et al. (1966) disclose the isolation and purification of *Aeromonas* aminopeptidase, and the action of the aminopeptidase on some di- and tripeptides. Prescott et al. (1966) do not disclose the removal of an N-terminal or N-terminal region alanyl residue from at least 48% of the polypeptide molecules contacted by the aminopeptidase and immobilization of the aminopeptidase as required by the claims.

The Office has failed to establish a *prima facie* case of obviousness, as the references, when combined, fail to teach or

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substrates on the hydrolysis of *Aeromonas* aminopeptidase.

[15] Prescott et al. (1975), p. 539, Table II, footnote b.

[16] Prescott et al. (1975), p. 538.

[17] Prescott et al. (1975), p. 540, Table III.

suggest each and every requirement of claims 22, 35, and 48.[18] Specifically, each of these references fails to teach or suggest cleavage of an N-terminal or N-terminal region alanyl residue from at least 48% of the polypeptide molecules contacted by the immobilized aminopeptidase. In fact, Blumberg et al., the only reference to specify a percent cleavage, discloses a mere 5.2% cleavage of N-terminal alanine from Cu_2-Zn_2 Human Superoxide Dismutase, as noted by the Office.[19] They also disclose negligible amounts of alanine as having been removed in Examples I and VI[20] - amounts less than those disclosed in the "non-removal" of Example X. Therefore, because the combination of references fails to teach or suggest all such claim requirements, the Office has failed to establish a *prima facie* case of obviousness.[21]

Moreover, the Office has failed to establish a *prima facie* case of obviousness, as there is no motivation contained within the references themselves to combine the references to achieve the claimed invention. Specifically, Blumberg et al. merely disclose that *Aeromonas* aminopeptidase ***may*** remove an alanyl group from the alanyl form of bGH, a mere 5.2% removal of an N-terminal alanyl from Cu_2-Zn_2 Human Superoxide Dismutase in Example X, and an even smaller amount of removal of alanine from Met-hGH and Met-Leu-hGH in Examples I and V. Pedersen et al. and Prescott et

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[18] MPEP §2142.

[19] *See*, present Office action dated June 17, 2003, at p. 3, lines 13-15.

[20] *See*, Blumberg et al., U.S. Patent No. 5,763,215 at column 9, Table I footnotes, and at column 14, Table V footnotes.

[21] MPEP §2142.

al. (1975) fail to disclose the removal of an alanyl residue from a polypeptide by the use of *Aeromonas* aminopeptidase. And Prescott et al. (1966) disclose a very poor relative rate of hydrolysis of alanine - a mere 0.7 for alanine versus, for example, 100 for leucine.

With such inconsistent results, one of ordinary skill would not be motivated by the above-mentioned references to attempt to remove an N-terminal or N-terminal region alanyl residue from a polypeptide using immobilized *Aeromonas* aminopeptidase. In fact, such disclosures teach away from the claimed invention, as they would seem to indicate that *Aeromonas* aminopeptidase cannot be used to cleave an N-terminal or N-terminal region alanyl residue from a polypeptide.

Moreover, the benefits obtained by immobilization of the *Aeromonas* aminopeptidase where neither expected from nor appreciated by the disclosures in above-mentioned references. The present specification discloses the removal of an N-terminal or N-terminal region alanyl residue from at least about 48% of the polypeptide molecules contacted by immobilized aminopeptidase, with percentages being as great as about 99.08%.[22] Blumberg et al. disclose a mere 5.2% removal of an N-terminal alanine, characterized by Blumberg et al. as a non-removal, and an even smaller amount of removal of alanine from Met-hGH and Met-Leu-hGH. Each of Pedersen et al., Prescott et al. (1975), and Prescott et al. (1966) fails to disclose the removal of an alanine residue and/or fail to disclose a percent cleavage. One of skill would not expect or appreciate the

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[22] *See*, for example, specification at p. 16, Table 1; p. 17, Tables 2 and 3; p. 18, Table 4; p. 19, Table 6; and p. 20, Table 7.

advantages of immobilizing *Aeromonas* aminopeptidase based upon the disclosures contained in these references. Accordingly, one of skill in the art would not be motivated to combine these references in an attempt to achieve the claimed invention.

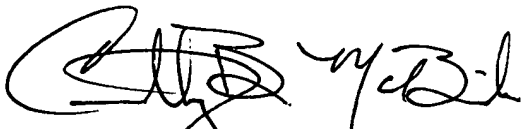
Claims 23-34 and 69-72, which depend from claim 22, claims 36-47 and 73-76, which depend from claim 35, and claims 49-60 and 77-80, which depend from claim 48, are patentable over Blumberg et al. and Pedersen et al. in view of Prescott et al. (1975) and Prescott et al. (1966) for the reasons stated above with respect to claims 22, 35, and 48 and by reason of the additional requirements which they introduce.

CONCLUSION

In light of the above arguments, Applicants respectfully request reconsideration and withdrawal of the rejection under 35 U.S.C. 103(a).

Applicants request an extension of time to and including November 17, 2003, for filing this amendment. The Commissioner is hereby authorized to charge any deficiency or credit any overpayment in connection with this amendment to Deposit Account No. 19-1345.

Respectfully submitted,



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